

Claims

1. A method of monitoring the temperature of a biochemical reaction, said method comprising effecting the reaction in the presence of a fluorescently labelled temperature probe DNA sequence which comprises a double stranded region which denatures at a predetermined temperature, the fluorescent label of said temperature probe sequence being arranged so that a detectable signal occurs at the point at which denaturation of the said region takes place; and monitoring fluorescence from said reaction mixture so as to determine when the said predetermined temperature has been reached.
2. A method according to claim 1 wherein the temperature probe DNA sequence comprises a labelled double stranded DNA sequence.
3. A method according to claim 1 wherein the temperature probe DNA sequence comprises a single nucleic acid strand, end regions of which hybridise together so as to form a loop or "hairpin" structure.
4. A method according to any one of the preceding claims wherein the fluorescent label comprises an intercalating dye.
5. A method according to claim 4 wherein the intercalating dye comprises SYBRGreen™ or SYBRGold™ or ethidium bromide.
6. A method according to any one of claims 1 to 3 wherein the fluorescent label used in the method of the invention may utilise fluorescence resonance transfer (FRET) as the basis of the signal.
7. A method according to claim 6 wherein the temperature probe DNA sequence is provided with a reporter and a quencher molecule, arranged so that the hybridisation of the strands

alters the spatial relationship between the quencher and reporter molecules.

8. A method according to claim 7 wherein the temperature
5 probe sequence is a single stranded sequence, where the end portions hybridise together and wherein the reporter molecule is attached in the region of either the 5' or the 3' end of the sequence and the quencher molecule is attached at the opposite end.
- 10 9. A method according to claim 8 wherein the reporter and quencher molecules are located on different strands of a DNA temperature probe sequence such that on hybridisation of the strands, they are brought into close proximity to each other.
- 15 10. A method according to claim 9 wherein FRET is established between an intercalating dye and a quencher molecule arranged on a strand of the temperature probe sequence such that it can absorb radiation from dye which is in close proximity on
20 hybridisation of the strands.
11. A method according to claim 7 wherein the temperature probe DNA sequences comprises a first DNA strand having a reporter molecule thereon, a second DNA strand having a
25 quencher molecule thereon, said first and second DNA strands being designed to hybridise to a third DNA strand such that the reporter and quencher molecules are brought into close proximity with each other.
- 30 12. A method according to any one of the preceding claims wherein the length of the temperature probe sequence is used to set the said predetermined temperature.
13. A method according to any one of the preceding claims
35 wherein the GC content of the temperature probe sequence is modified to obtain the desired predetermined temperature.

16. A method according to claim 15 wherein the length of the temperature probe sequence is similar to that of an amplicon of the PCR reaction.